Absorption of Arsenate and Arsenite by Arsenic Hyperaccumlating Fern (Pteris vittata)

V. 4. Absorption of Arsenate and Arsenite by Arsenic Hyperaccumlating Fern (*Pteris vittata*)

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Phytoremediation using an arsenic hyperaccumulator, *Pteris vittata* L., has generated increasing interest worldwide, for it's both environmentally sound and cost effective. However, the mechanisms of arsenic uptake and accumulation by this plant are not clear at this time. This study shows the uptake of arsenate (As(V)) and arsenite (As(III)) using a hydroponic culture of *P. vittata*. The fern takes up both As(V) and As(III) from the culture which spiked with 50 mg of arsenic per litter, and can grow within 5 days of experimental period. Final amount of arsenic accumulated in the fern is 3.2 mg (As(V)), and 3.8 mg (As(III)). The submilli-scale distribution of elements by PIXE analysis clearly shows the accumulation of arsenic in fronds of *P. vittata.*

Introduction

Arsenic contamination of soils and groundwater from various sources such as mine and urban wastes, wood preservatives and pesticides is of great environmental problem[1,2)]. A number of technologies for arsenic-contaminated soils have been proposed. However, most of them are generally expensive and may produce secondary wastes. Phytoremediation is an acceptable technology for relative lower level of arsenic contaminated soil because it is recognized cost-effective and environmental friendly process. Recently, *Pteris vittata* L., a kind of fern, has been found as an arsenic hyperaccumulator plant[3)]. This species can accumulate up to 20 g/kg of arsenic in its above ground biomass. Recently, several other fern species have been reported to have ability to hyperaccumulate arsenic similar to *P. vittata*[4)]. In case of *P. vittata* grown on arsenic-contaminated soil, most of the arsenic localized to the vacuoles in epidermal cell of fronds[3,5)]. It is undoubtedly that arsenic enters in the roots and translocate to the fronds. Arsenic accumulated in the frond of *P.*

vittata is mostly arsenite (As(III)) form, while arsenic in the root occurs predominantly arsenate (As(V)) form[6]. It is believed that arsenate taken up by roots is converted to arsenite before or after it is translocated to the frond[7]. The entry system of arsenic to *P. vittata* is not studied well. Recently, Poynton et al.[8] showed that arsenate influx into the roots of hyperaccumlating ferns is greater than in nonhyperaccumulating ferns and speculated that the phosphate transporting protein has a responsibility for the influx. However, in our knowledge, there are no report concerning to arsenite influx into hyperaccumlating ferns. The aim of this work is to show the uptake of arsenate and arsenite into the *P. vittata* using chemical and PIXE analysis.

Materials and Methods

Plant material

P. vittata L.[3] was used throughout the experiment. Fern seedlings were used 3 months after spore germination. The roots were washed carefully in tap water to remove soil particles. The seedlings were then transferred to hydroponic water containing nutrient solution. To compare the effect of phosphate, phosphate-free nutrient solution was also used for several hydroponic experiments. The nutrient solution was aerated continuously. Seedlings were grown on the solution for 1 week in a growth camber with a 16 hours light period, 25 degree/20 degree day/night temperature and 70% relative humidity.

Arsenate and arsenite uptake experiments

After 1 week cultivation in hydroponic solution, the roots were washed well in tap water. Each seedling was placed in 100 ml conical beaker filled with 100 ml uptake solution. Then arsenic in the form of either As(V) as sodium arsenate ($Na_2HAsO_4\,7H_2O$), or As(III) as sodium arsenite ($NaAsO_2$) was added to the uptake solution to be final concentration 50 mg-As per litter. The seedlings treated were incubated in the growth camber at the same condition for a day. After 24 hours incubation, total weight of the beaker was measured to determine the amount of transpiration by the fern. Then the roots were washed and the seedling was placed new conical beaker filled with 100 ml of fresh solution containing the same kind and amount of arsenic. This cycle was repeated for 5 days. Arsenic concentration of the uptake solution was determined by ICP-MS (Hewlett Packard, HP-4500). Distribution image of elements in the plant sample was analyzed by submilli-PIXE camera equipped in the Dynamitron laboratory of Tohoku University. Details of the system and analytical methods are described previously[9,10].

3. Results and Discussion

Figure 1 shows a time course of arsenic concentration in hydroponic culture solution. Concentration of arsenic is obviously decreased from the initial concentration especially in the arsenite treatment at the first day while the concentration of arsenic is same in both of arsenate and arsenite treatment after 2 days. Although about 10% of the culture solution was decreased per day by transpiration, the arsenic concentration in the solution is not changed to the initial value in both cases. This result means that most of arsenic, both arsenate and arsenite, are transferred into the plant from root with a flow of water absorption. Figure 2 shows cumulative curve of arsenic in biomass of *P. vittata*. Final amount of arsenic accumulated in the fern is 1,500 mg per kg (wet weight) of the plant biomass in the arsenite treatment and 1,100 mg per kg in the arsenate treatment after 5 days. These values well agree to total amount of arsenic accumulated in the plant determined by whole plant analysis (data not shown). Based on these results it is concluded that arsenite influx into the roots of *P. vittata* is same as or greater than arsenate influx.

Submilli-PIXE analysis was applied to detect the distribution of arsenic in the plant. Figure 3 shows the distribution of arsenic in frond of *P. vittata* using submilli-PIXE camera. Arsenic was concentrated especially to near edge of the frond in both of arsenate- and arsenite-containing hydroponic culture. There is no significant difference for the distribution of arsenic in these two cases. On the other hand, it is difficult to detect the arsenic in root by submilli-PIXE analysis (data not shown). These results indicate that once arsenic, both arsenate and arsenite, enter from the medium, it translocates quickly from root to frond. Arsenic distribution image of a frond of *P. vittata* grown with or without phosphate are shown in Fig. 4. There is no effect of phosphate ion on arsenic accumulation so the entry system of arsenic to *P. vittata* must be proposed.

4. Conclusion

- Both arsenite and arsenate transferred into *P. vittata*.
- Phosphate does not effect on arsenic accumulation of *P. vittata*
- Arsenic was stored in frond of *P. vittata*, especially concentrated near edge.

Acknowledgements

This research was partially supported by Grant-in-Aid for Science Research from the Japan Society for Promotion Science (16656277).

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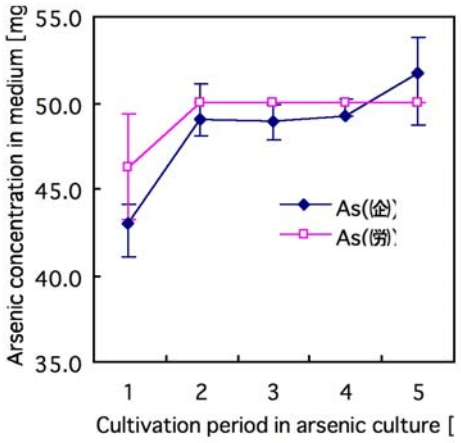

Figure 1. Time course of arsenic concentration in culture solution

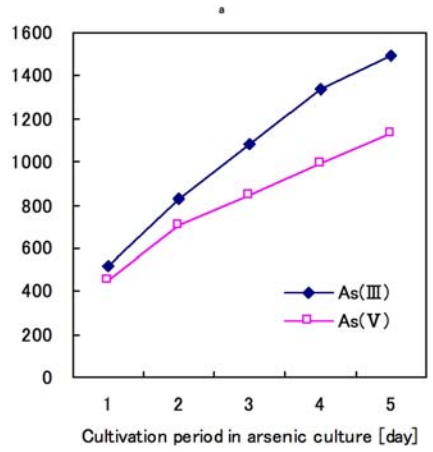

Figure 2. Cumulative curve of arsenic in biomass of *P.vittata*

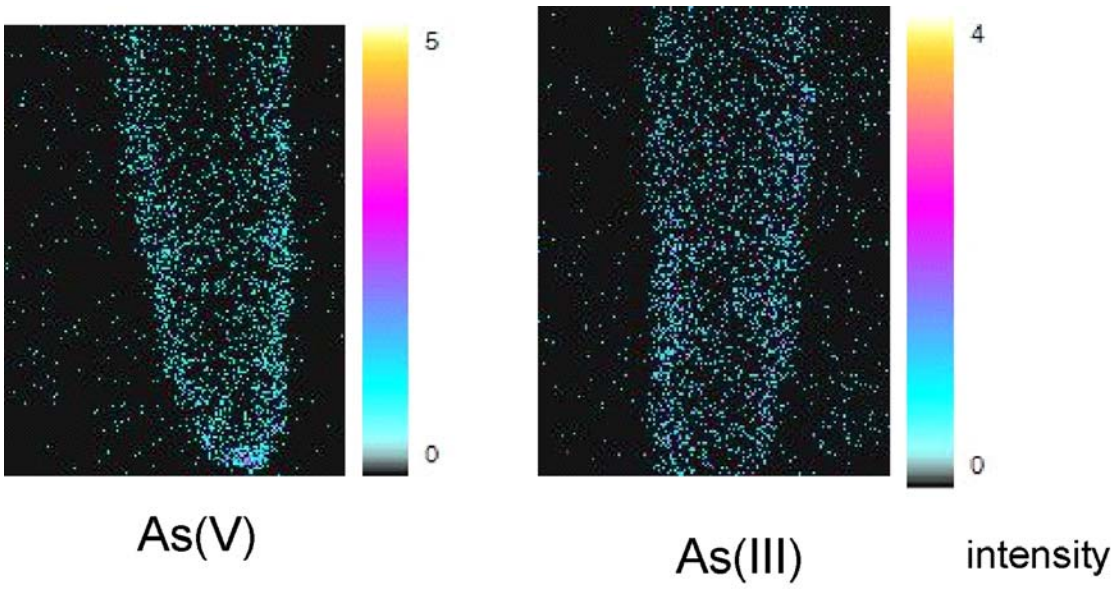

Figure 3. Submilli-PIXE dot-map of arsenic in a frond of *P. vittata* grown in an arsenate- or arsenite-containing hydroponic culture.

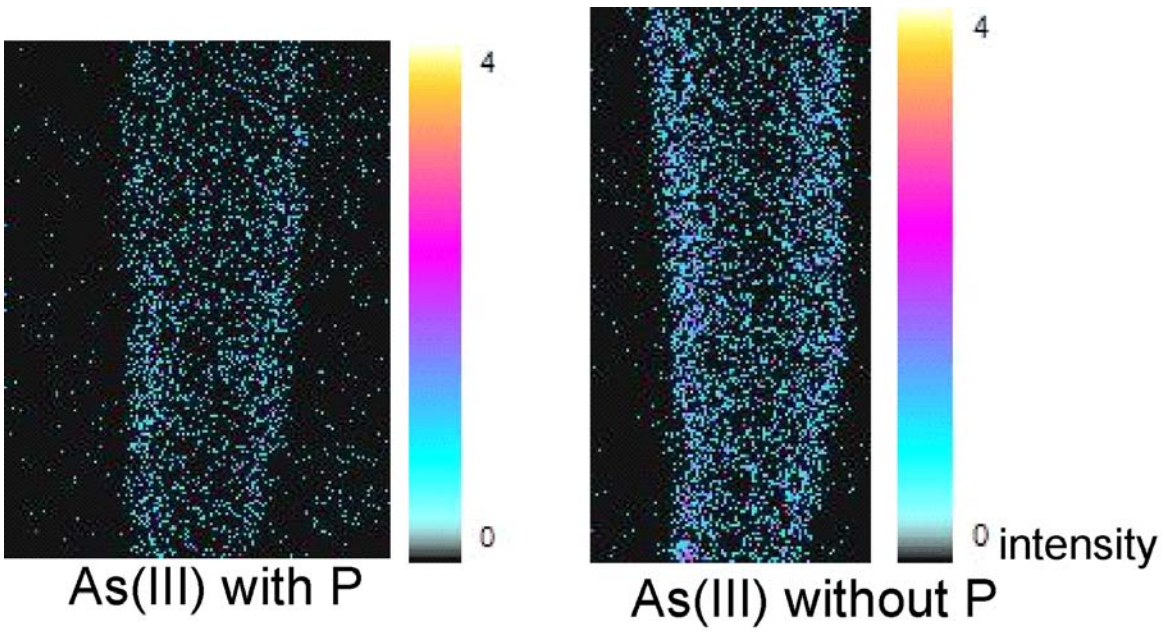

Figure 4. Submilli-PIXE dot-map of arsenic in a frond of *P. vittata* grown with or without phosphatecontaining hydroponic culture.